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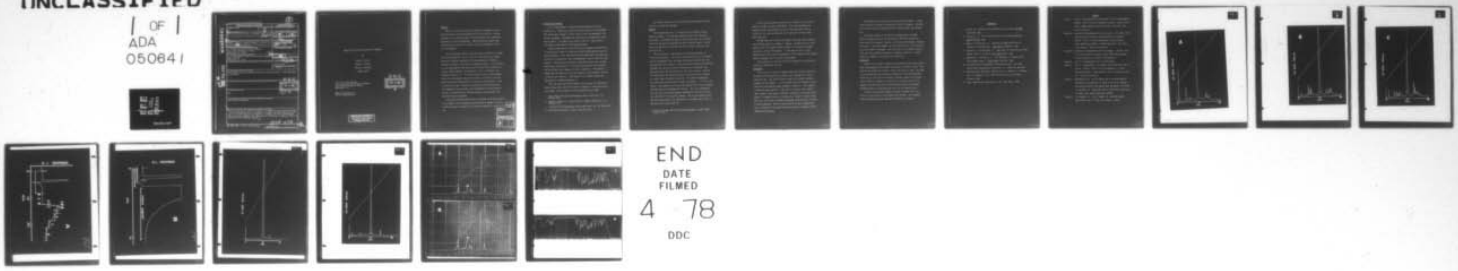

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The purity of several brands of eugenol was compared by using High Performance Liquid Chromatography with a UV detector. Greater than 95% of the impurities seen in the USP eugenols were removed by preparative liquid chromatography. NMR spectroscopy suggests that there may be a difference in chemical reactivity between purified and stock eugenol.

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ANALYSIS AND PURIFICATION OF EUGENOL

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SYNOPSIS

The purity of several brands of eugenol was compared by using High Performance Liquid Chromatography with a UV detector. Greater than 95% of the impurities seen in the USP eugenols were removed by preparative liquid chromatography. NMR spectroscopy suggests that there may be a difference in chemical reactivity between purified and stock eugenol.

Zinc oxide/eugenol cements are widely used in dentistry as temporary filling materials, cavity liners for pulp protection, capping materials, temporary cementation of fixed prostheses, impression materials, and major ingredients of endodontic sealers. Early studies on zinc oxide/eugenol have shown that the mixture was the least irritating, common temporary filling material, and is, therefore, the material of choice.[1,2,3] In recent studies, Erausquin and Muruzabal[4] found zinc oxide eugenol cement to be highly irritating to the periapical tissues and caused necrosis of bone and cementum when it came in contact with them. Braunstrom and Nyborg[5] found hyperemia and cell aggregation resulted when zinc oxide/eugenol paste was applied to very thin dentinal walls; and when eugenol alone was used, the reaction was more pronounced.

Since these previous studies did not indicate the grade or purity of the eugenol, this investigation was designed to compare the purity of several brands of eugenol and to develop a method for its purification.

MATERIALS AND METHODS

U.S.P. Grade Eugenol[6] (> 95% purity) was obtained from three manufacturers. Quantitative analysis was carried out on two μ-Bondapak C18 reverse phase columns in series using a liquid chromatograph* with solvent programmer. Absorbance was monitored on a UV detector at both 254 and 280 nm. The sample was eluted by a 30 min. linear gradient from 25/75 to 100% acetonitrile**/deionized water.

Optimum conditions for the purification of eugenol were determined on an analytical μ-porisil column using a 60/40 CH_2Cl_2/Hexane mixture to elute the sample. After the conditions were established, the sample was purified on a preparative liquid chromatograph† using a refractive index detector. Fractions were collected to determine the purity at various times during the separation. The purest fractions were then combined and the solvents were removed on a rotary evaporator. The combined samples were analyzed for purity and subjected to spectroscopic analysis to confirm their identity. Confirmation of the eugenol samples was determined on an NMR spectrometer.††

Both the stock and purified eugenols were prepared for NMR spectoscopy by preparing a 20% (V/V) solution of eugenol in deuterated chloroform ($CDCl_3$) with the addition of 5% tetramethylsiliane (TMS).

* Waters Model 244 Liquid Chromatograph, Waters Associates, Inc. Milford, Mass.

** Burdick & Jackson U.V. Grade, Burdick & Jackson Laboratories, Inc., Muskegon, Mich.

† Prep 500 Liquid Chromatograph, Waters Associates, Inc., Milford, Mass.

†† EM-360 NMR Spectrometer, Varian, Palo Alto, Calif.

For further comparison, the stock and purified eugenols were subjected to infrared spectroscopy‡.

RESULTS

The chromatograms (Fig. 1) illustrate the differences between brands of eugenol. The main peak eugenol can easily be detected in each sample. No attempt was made to identify each peak, but different impurities can easily be detected for each individual sample. The UV spectra at 280 nm also indicated that the different brands had their own impurities.

The optimization of conditions on normal phase resulted in a K'=3.6 for the eugenol. The initial run purified only 5 ml of eugenol, and its chromatogram (Fig. 2A) shows the various fractions that were collected for purity analysis. Purity analysis of the various fractions indicated that the third fraction collected still contained a few impurities which were not detected in the fourth fraction. Fraction six remained pure but beyond this point the concentration of eugenol vs. solvent made collection impractical. The second chromatogram (Fig. 2B) shows the purification of 30 ml of eugenol. In this run the column is overloaded but the resolution remained sufficient to separate the impurities of the compound of interest. The final chromatogram (Fig. 3) shows the purified eugenol (combination fractions 4-6) with more than 95% of the impurities removed. This was calculated by comparing the peak areas of the impurities in the sample before (Fig. 1) and after purification (Fig. 3).

‡ Perkin Elmer Model 283 Infrared Spectrophotometer, Perkin Elmer, Norwalk, Conn.

The purification process resulted in an increase in purity of the eugenol from 95% to greater than 99.75%. The purified eugenol was reanalyzed after four months (Fig. 4) and there was found to be an increase in the impurity peaks but not to the level of the original stock solutions.

The NMR spectra of the purified eugenol (Fig. 5B) closely matched the published spectra for eugenol.[7] However, the NMR spectra of stock eugenols (Fig. 5A) showed a marked decrease in the phenolic hydrogen peak seen in the purified eugenol. It was observed that in stock eugenol spectra there were no concomitant shifts produced by the missing phenolic hydrogen peak in the other proton peaks.

The IR spectra of the stock and purified eugenol did not show any significant differences (Fig. 6).

DISCUSSION

The three U.S.P. brands of eugenol show some different impurities with many of the peaks being the same in each compound with similar retention times. The similar impurity peaks are probably break-down products from the eugenol. It is the peaks that are different in each brand that could possibly cause problems in dentistry. This suggests a reason why some studies[1,2,3] show little irritation while others[4,5] report eugenol to be highly irritating. According to the Merck Index[8] eugenol is prepared from oil of cloves which is known to contain small quantities of furfural, and methyl amylketone which are known irritants of mucous membranes. While no identification of these compounds was made, elimination of the impurities would minimize the chance of these compounds being present.

The method of purification was relatively easy and rapid. It took fifteen minutes to perform the separation of 30 ml of eugenol. Over 95% of the impurities were removed producing a product greater than 99.75% pure.

The marked decrease in the phenolic hydrogen peak in the NMR spectra suggests that there is some chemical difference between the stock and purified eugenol; however, the IR spectra indicated no chemical differences. This would suggest that sole alteration is in the dissociation of the phenolic hydrogen. This alteration in the eugenol molecule could indicate a possible difference in chemical reactivity. Further studies are needed to investigate this possibility.

CONCLUSION

Several U.S.P. brands of eugenol were analyzed for purity by reverse phase chromatography and different contaminants were seen in each. This could be one reason for different results from study to study. Over 95% of the contaminants were removed by preparative liquid chromatography, resulting in a final purity of 99.75%. Previous studies on the pathological effects of eugenol on tissue did not indicate the purity of eugenol, but the use of this ultra-pure eugenol should provide good standardization for experimentation which will be reported later.

Finally, even the pure eugenol was shown to degrade with time; but not to the extent of the original stock solution. This suggests that a possible expiration date may be necessary for eugenol.

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LEGENDS

Figure 1. H.P.L.C. chromatograms of three brands of U.S.P. Grade Eugenol. Columns: 30 cm X 3.9 mm μ-Bondapak C_{18}, eluent: acetonitrile/water; linear gradient, 30 min; flow rate 2.0 ml/min. See text for details.

Figure 2A. Preparative chromatogram of 5 ml of eugenol. The numbers indicate the various fractions collected for purity analysis. Columns: Two 5.7 X 30 cm PrepPak-500 silica cartridges, eluent 60/40 CH_2Cl_2/Hexane, flowrate 250 ml/min, refractive index detector. See text for details.

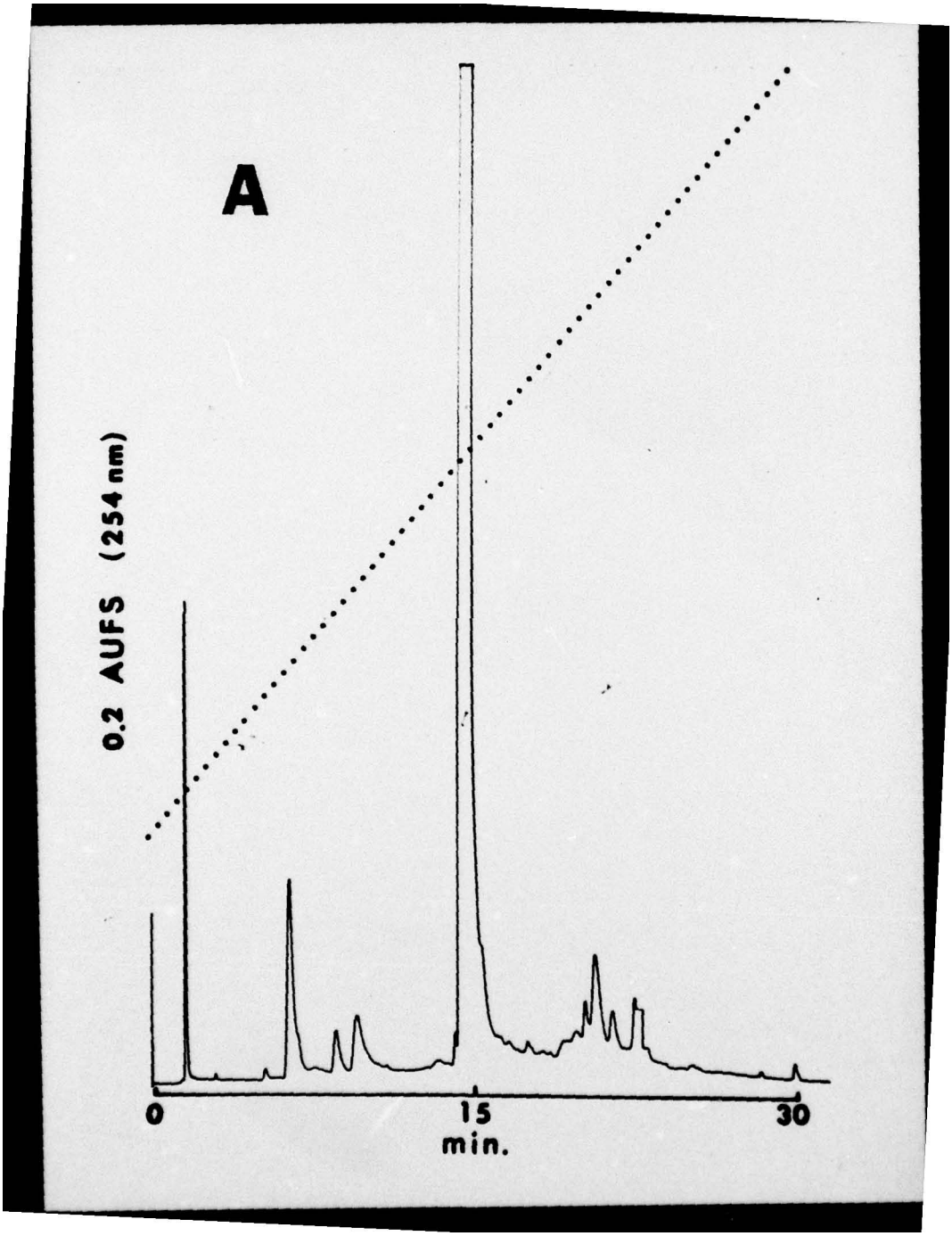
Figure 2B. Preparative chromatogram of 30 ml of eugenol. Columns: Two 5.7 X 30 cm PrepPak cartridges, eluent 60/40 CH_2Cl_2/Hexane, flowrate 500 ml/min, refractive index detector.

Figure 3. H.P.L.C. chromatogram of the purified eugenol.

Figure 4. H.P.L.C. chromatogram of the eugenol four months after purification. Columns: 30 cm X 3.9 mm μ-Bondapak C_{18}, eluent: acetonitrile/water; linear gradient, 30 min; flowrate 2.0 ml/min. See text for details.

Figure 5. NMR spectra of A. U.S.P. Eugenol and B. purified eugenol. The phenolic hydrogen peak is indicated by -OH at 5.59 ppm. The concentrations of the samples were 20% eugenol and 1% TMS in chloroform -d. Sweep time 10 min, sweep width 10 ppm, end of sweep 0 ppm, sample temperature ambient.

Figure 6. I.R. spectra: A. U.S.P. Eugenol, B. purified eugenol. NaCl plates; scan: 12 min; slit program: normal.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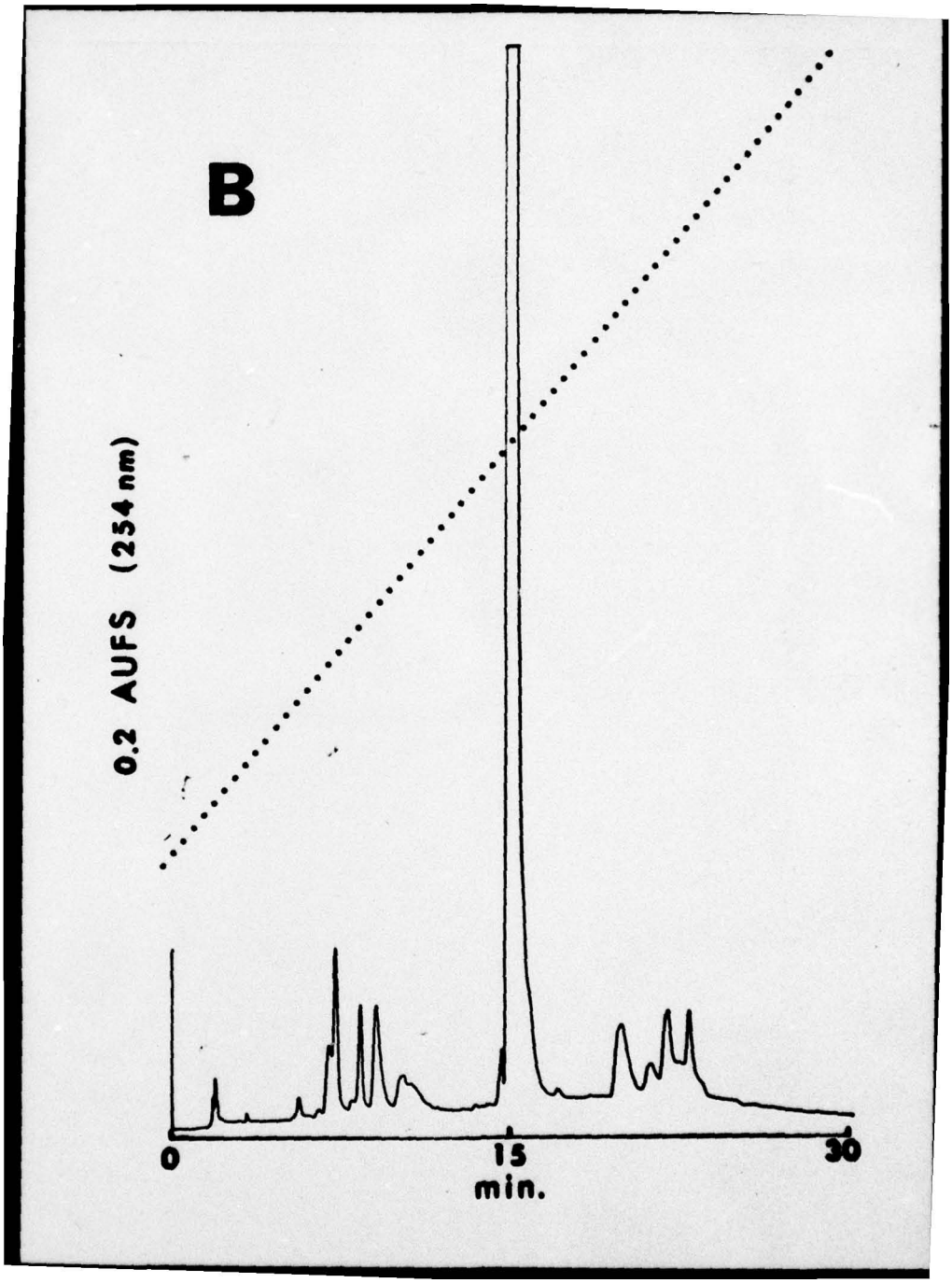
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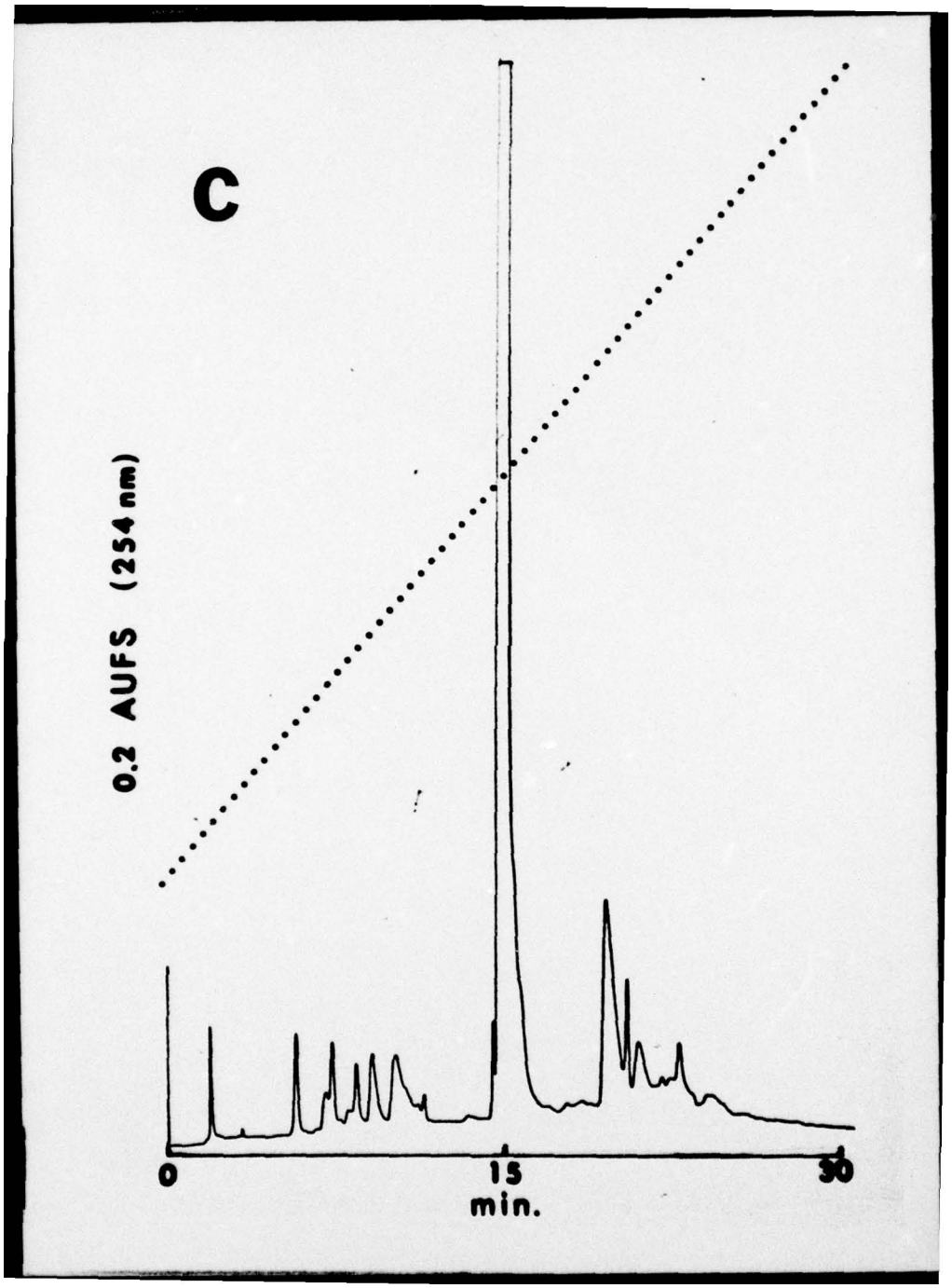
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Fig. 2
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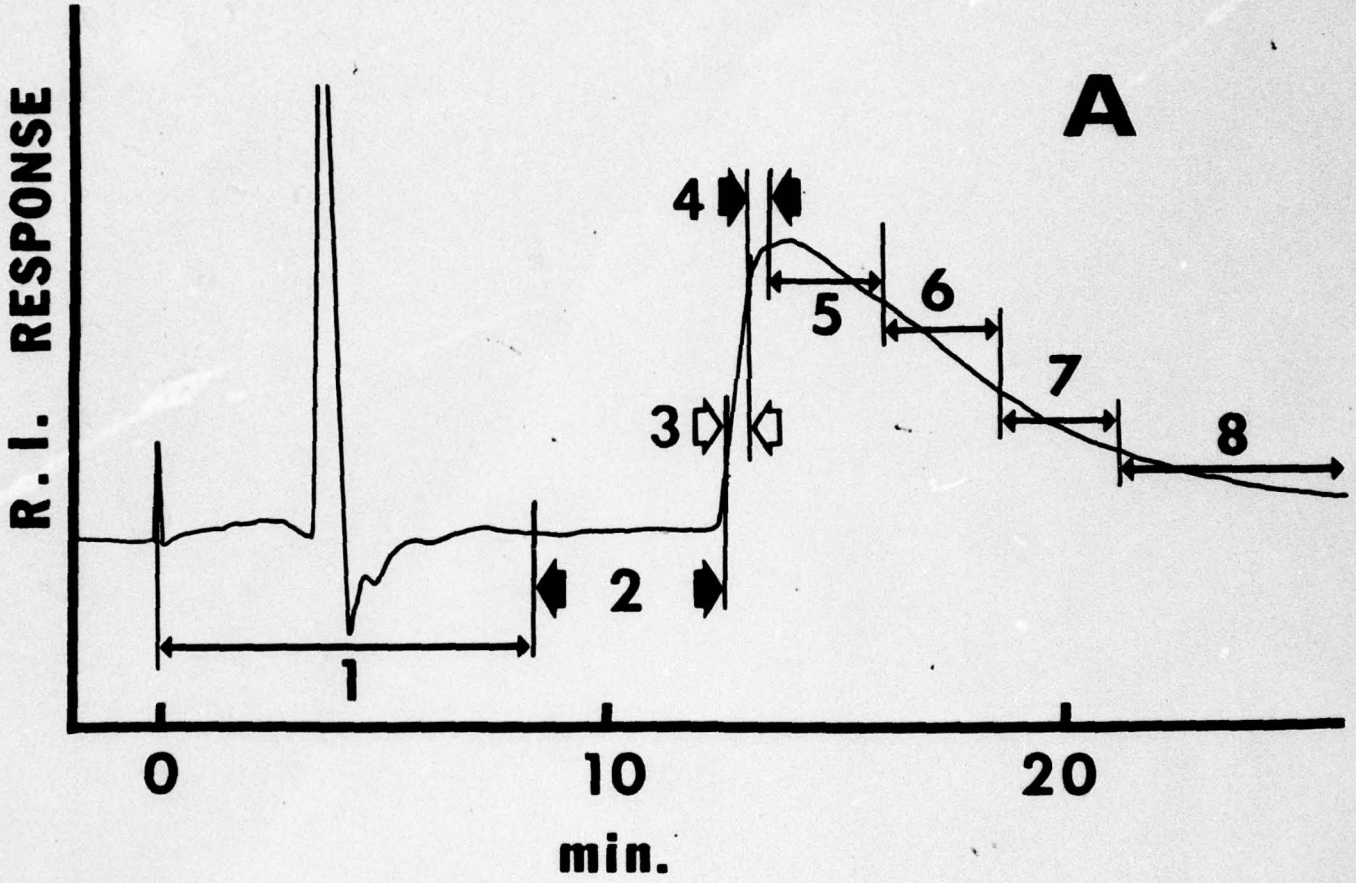
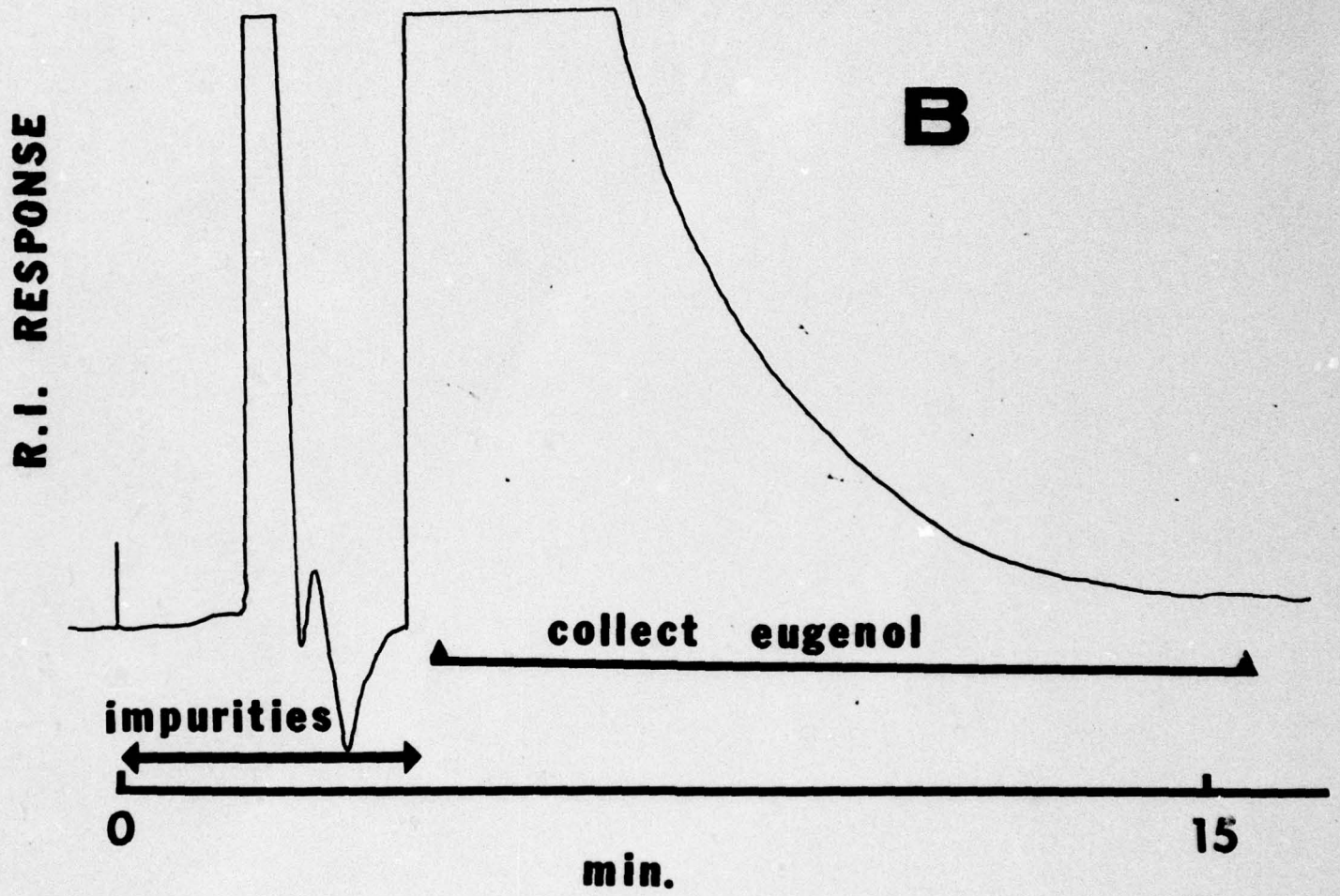


Fig. 2
B



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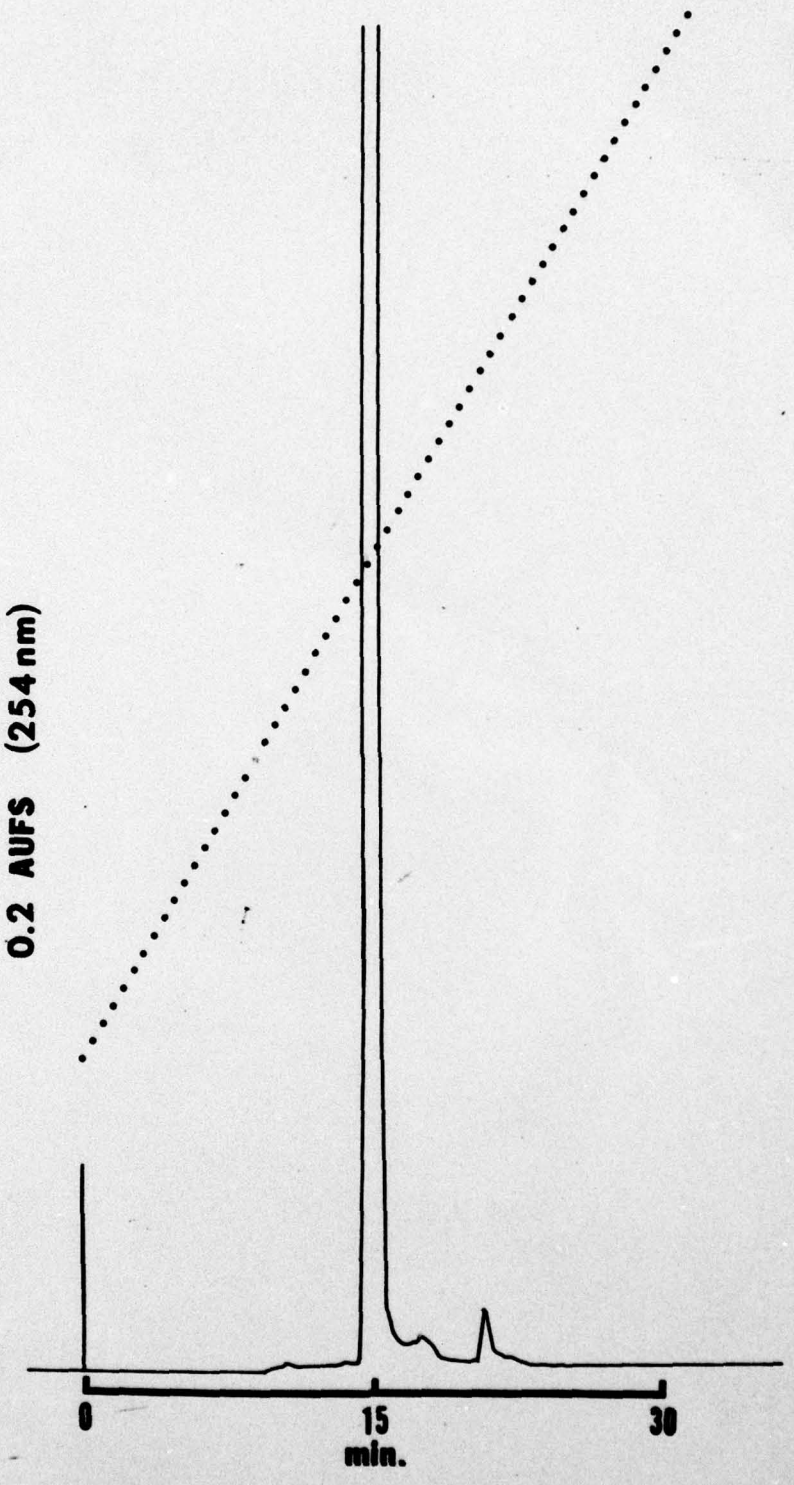
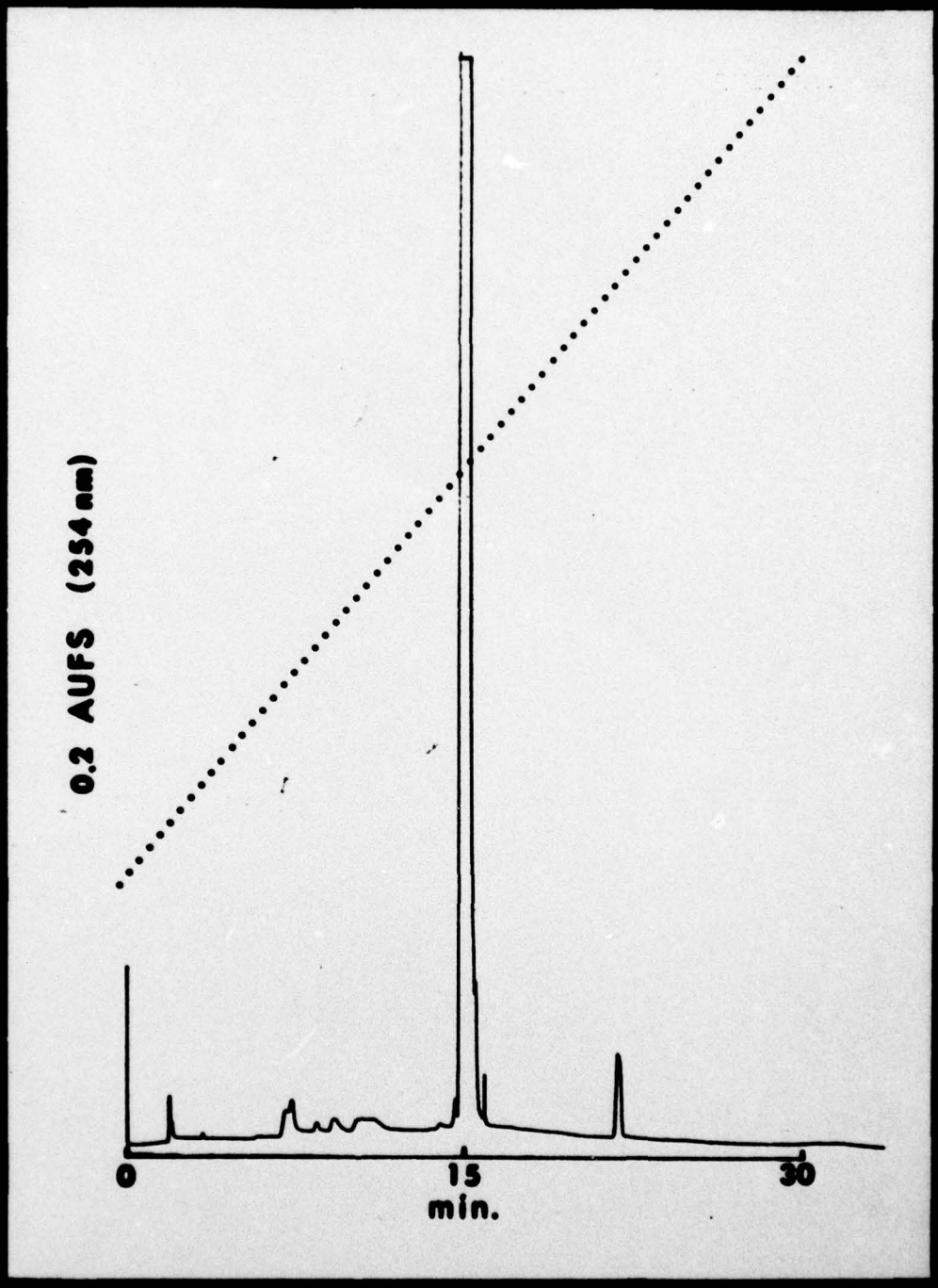


Fig. 4



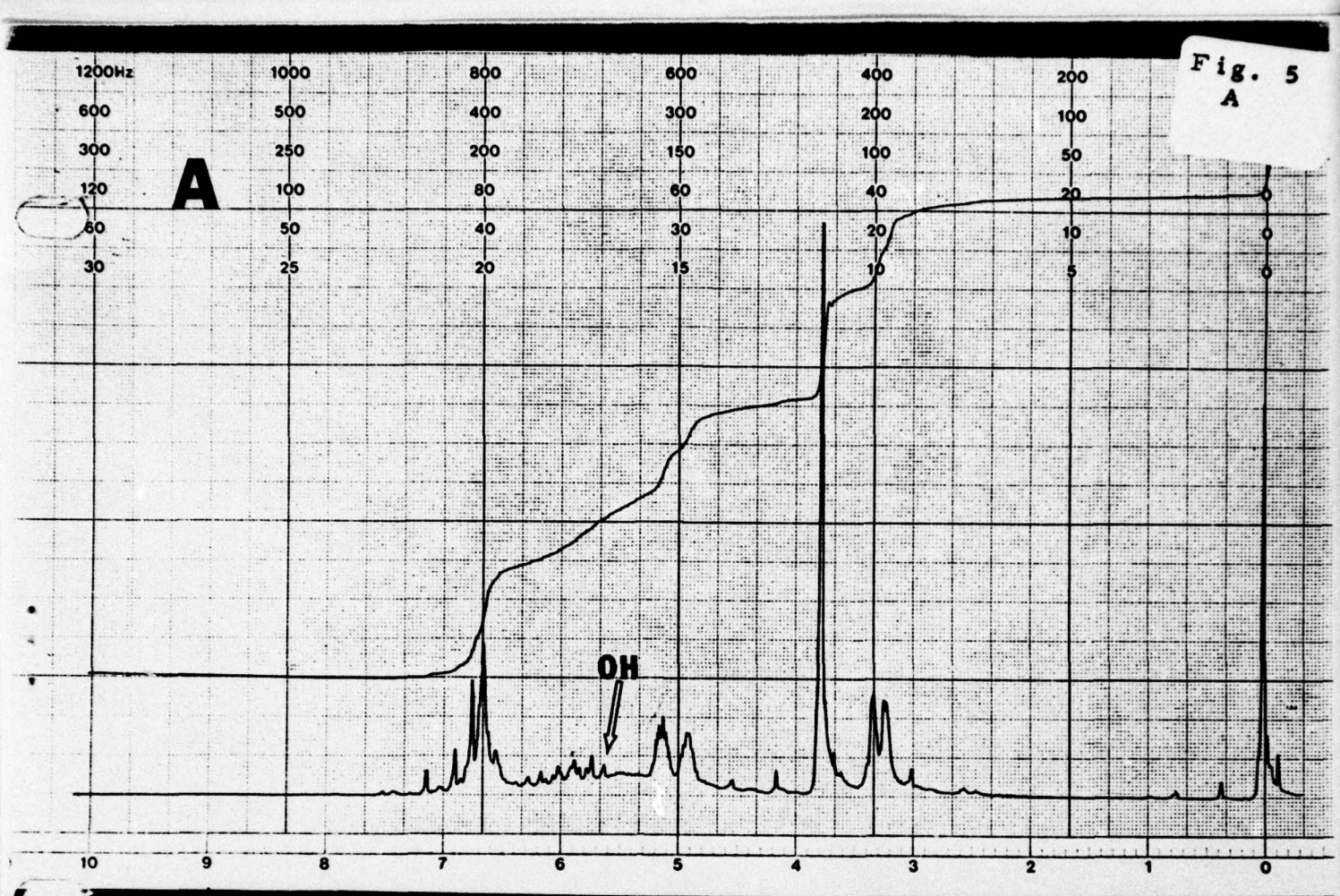

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Fig. 5
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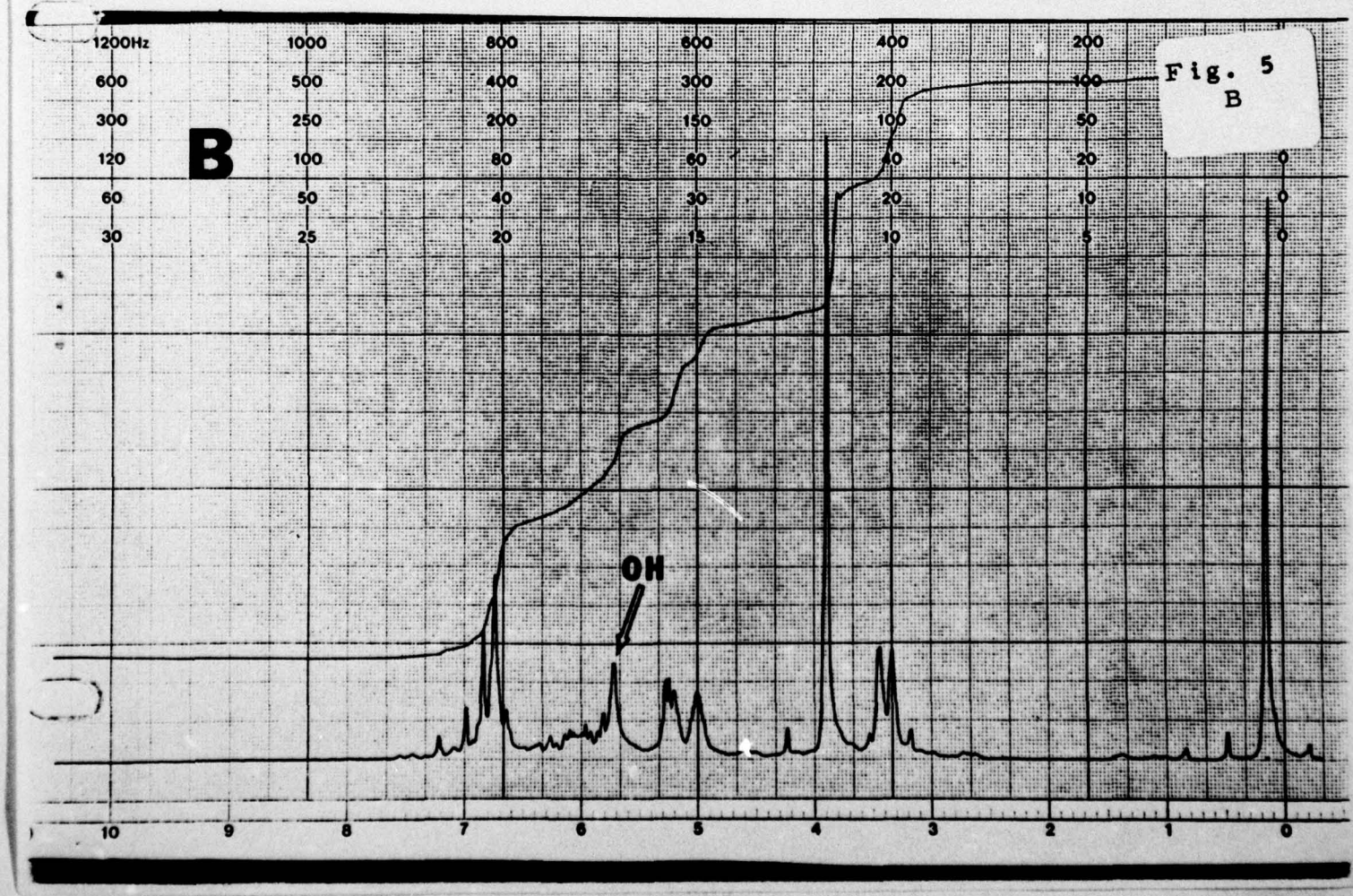
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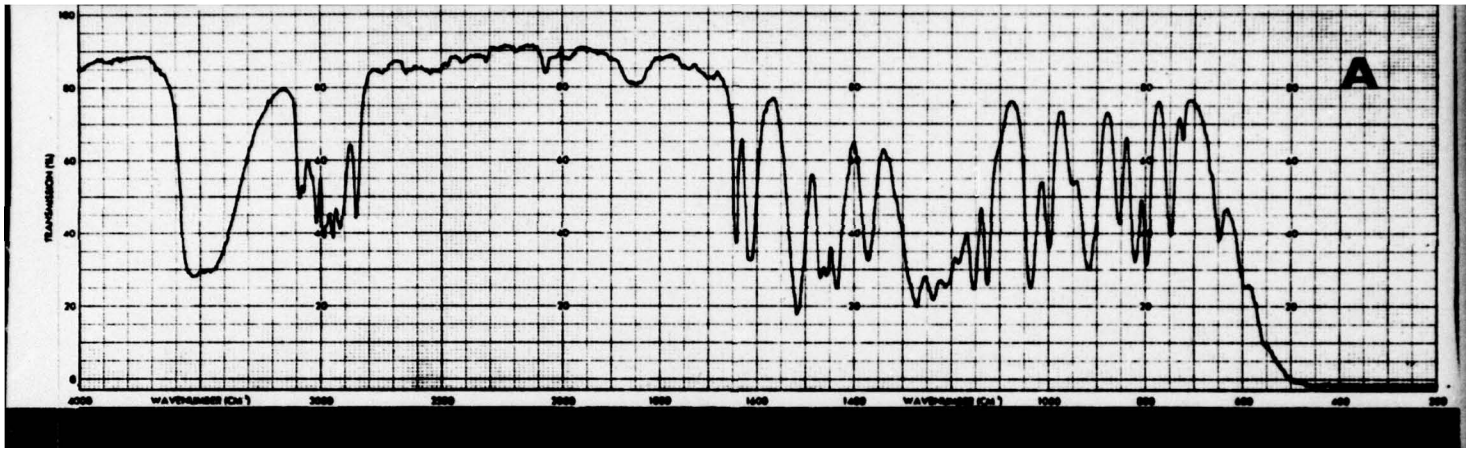
Fig. 5
B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